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Olfactory Coding: Tagging and Tuning Odor-Activated Synapses for Memory

A recent study in the locust olfactory system shows how neuromodulators can alter the rules of synaptic plasticity to form associative memories through the use of 'tagged' synapses.

Zane N. Aldworth and Mark Stopfer

Scents evoke vivid recollections — the smell of sunscreen brings the ocean to mind; a whiff of perfume calls forth a long-ago friend. It seems effortless to form and remember powerful connections between odors and other sensory stimuli. Yet, a physiological understanding of how our brains instantiate these associations remains elusive. Hebb famously suggested [1] that a synapse could be strengthened when the presynaptic and postsynaptic neurons are activated together. The discovery of spike-timing-dependent plasticity (STDP) [2], a process that can either increase synaptic strength (when the presynaptic cell is activated milliseconds before the postsynaptic cell), or decrease synaptic strength (when the timing is reversed), provided a physiological mechanism for this plasticity. STDP has been shown to occur in many species, including the locust [3].

Connecting Hebbian STDP to the formation of memory, however, has been surprisingly difficult, partly because STDP operates at much shorter time scales than the behavioral experiences that lead to new memories. For example, a recent study showed that in moths, animals that readily learn to associate odors with a tasty reward, odors reliably evoke spiking in Kenyon cells, neurons long believed to help encode odors and form memories. But the odor-evoked spiking was ephemeral; it ended several seconds before a rewarding drop of sugar water was presented to the animal, long after the millisecond-scale time window for STDP had closed [4]. Behavioral tests showed this training procedure induced new memories, but because pre- and post-synaptic spiking linking odor and reward could not occur in these cells with the required timing, STDP alone could not be responsible for forming them. How to resolve this dilemma? An elegant new study by Cassenaer and Laurent [5] points to a solution: STDP can 'tag' an odor-activated synapse, signifying and sustaining its identity until the reinforcement signal arrives.

Because the insect olfactory system is relatively simple and accessible it has become a useful model for the study of sensory processing and associative memory [6]. Odors are transduced by odorant receptors and their associated olfactory receptor neurons in the antennae (Figure 1A) [7]. These afferent neurons carry information to the antennal lobe, where lateral interactions among the receptor neurons, local neurons and projection neurons rearrange odor-evoked responses into temporally structured patterns of spiking distributed across groups of projection neurons. These firing patterns are also segmented into a sequence of time bins by ~20 Hz oscillations generated in the antennal lobe [8–11]. Intensely spiking projection neurons carry this information from the antennal lobe to the calyx of the mushroom body, where huge numbers of Kenyon cells respond to the odor sparsely with spikes that are few and far between [12,13]. Kenyon cells are influenced by the oscillatory patterning generated in the antennal lobe [9] and transmit the oscillations to postsynaptic targets, including the mushroom body's β-lobes [14].

Cassenaer and Laurent [3] had previously shown that STDP can occur at the synapse between Kenyon cells and β-lobe neurons in the locust. This first demonstration of STDP in an invertebrate showed that STDP acts here as a homeostatic mechanism, maintaining the integrity of the oscillatory signal passed along from the antennal lobe, rather than as a mechanism of memory. But in their new work, Cassenaer and Laurent [5] returned to the Kenyon cell–β-lobe neuron synapse to explore whether STDP there can be mnemonic. The authors first characterized responses of β-lobe neurons to a range of odorants believed relevant to behaving locusts. Consistent with previous studies [3,14], the authors found that the temporally patterned spiking responses of β-lobe neurons varied with the odor, and that firing rates of the β-lobe neuron vastly exceeded those of their presynaptic Kenyon cells. The authors also found individual β-lobe neurons responded much less selectively to odorants than did individual Kenyon cells, and that their

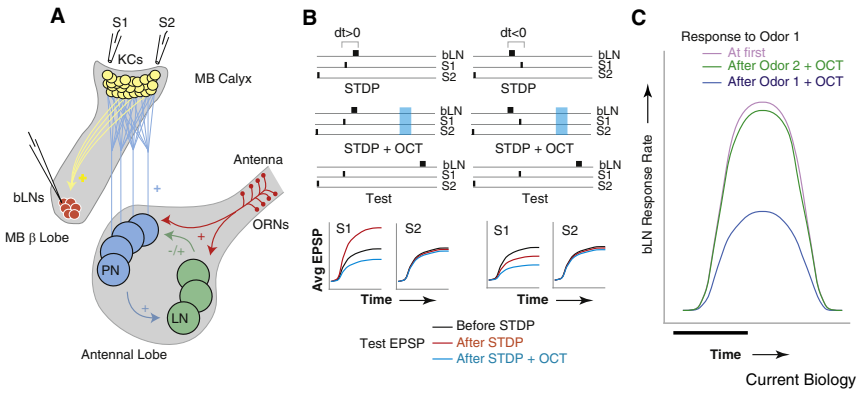

Figure 1. Neuromodulation of STDP in the insect olfactory system.

(A) The locust olfactory system receives sensory input from olfactory receptor neurons (ORNs) mainly on the antennae. These neurons synapse with both local neurons (LNs) and projection neurons (PNs) in the antennal lobe (AL), the first olfactory center within the brain. The PNs alone carry olfactory information to other areas of the brain, including the Kenyon cells (KCs) of the mushroom body calyx (MB calyx), which in turn synapse on the β-lobe neurons (bLNs). (B) Spike-timing-dependent plasticity (STDP) was elicited at the KC–bLN synapse. Two well-separated groups of KCs in the mushroom body (MB; S1 and S2, one for pairing, one for control) could be activated extracellularly, and individual bLNs could be activated intracellularly. To elicit STDP, stimuli to KCs and bLNs were paired, forward (dt > 0) or backward (dt < 0), within narrow temporal windows (±30 ms, upper sets of traces). Induction of STDP was sometimes followed by injection of octopamine (OCT) into the β lobe (middle trace). The results of these manipulations were tested afterward (bottom traces): STDP facilitated the KC-elicited response in the bLN (rising phase of the EPSP is shown) when dt > 0, and diminished the response when dt < 0; delayed delivery of OCT decreased EPSPs elicited only at STDP-tagged synapses (S1). (C) In a more naturalistic test, firing in the bLNs elicited by an odor (odor 1) was reduced after that odor specifically (odor 1, but not odor 2) had been paired with OCT injection into the β-lobe.

spikes occurred at a favored phase position of the oscillatory cycle.

To test whether these responses were consistent with known properties of the mushroom body circuit, Cassenaer and Laurent [5] devised a computational model including connectivity and STDP rules known to exist between Kenyon cells and β-lobe neurons. The model gave mixed results: it could reproduce the phase of firing seen in β-lobe neurons *in vivo*, but could not reproduce response probability or the extent of response saturation across the population of β-lobe neurons. This mismatch between model and brain suggested a page was missing from the STDP rulebook. The authors thus launched a set of experiments to learn more about the β-lobe neurons. Simultaneous intracellular recordings from pairs of β-lobe neurons revealed something new: about one of every four pairs was interconnected by inhibitory synapses. Adding lateral inhibition to the model brought its results into close agreement with experimental data and, further, showed that the β-lobe neurons maximize their available dynamic range for coding odors.

But could STDP contribute to associative memory? To test this Cassenaer and Laurent [5] delivered current pulses extracellularly to elicit spikes in presynaptic Kenyon cells and intracellularly to elicit spikes in β-lobe neurons (Figure 1B). By varying the timing of these paired stimuli, the authors confirmed the STDP rules they had previously characterized in the locust. Now they added a new ingredient: the neuromodulator octopamine, which is believed to be released throughout the insect brain as a reward signal when the animal consumes an appetitive stimulus like sugar water [15]. Remarkably, injecting a tiny squirt of octopamine into the β-lobe one second after an STDP pairing (stimulation at site S1 in Figure 1A,B) caused a reliable decrease in the size of the response that Kenyon cell activation triggered in the β-lobe neuron. This decrease occurred whether the timing of the Kenyon cell–β-lobe neuron pairing would otherwise have led to increased or decreased synaptic strength in the absence of the neuromodulator. A control procedure in which octopamine was delivered, but in which a different population of Kenyon cells was activated at delays much longer than the STDP window (stimulation at site S2 in Figure 1A,B) did not affect synaptic strength. These results revealed a new property of STDP: even though octopamine had spread throughout the β-lobe, its neuromodulatory effect occurred only at synapses that had been 'tagged' earlier by STDP.

Cassenaer and Laurent [5] found the STDP–octopamine modulation could take place when the β-lobe neurons were activated by odorants rather than by current injection: odor-evoked firing rates of β-lobe neurons dropped significantly after that odor had been paired with octopamine injection (Figure 1C). This reduction reflected a specific decrease in synaptic strength that the modulator induced between STDP-tagged presynaptic Kenyon cells and β-lobe neurons; a control experiment in which other odorants were paired with the octopamine injection did not show this reduction. Since the connections β-lobe neurons make with other β-lobe neurons are inhibitory, the authors suggested the decrease in spiking associated with octopamine reinforcement would cause the as-yet unidentified postsynaptic targets of tagged β-lobe neurons to increase their odor-evoked spiking.

Together, these results could resolve the timing mismatch exemplified by the experiments in moths [4]: octopamine release, even if delayed beyond the narrow STDP window, could specifically modulate those odor-specific synapses earlier tagged through STDP. By changing the rules of STDP, octopamine could allow a well-described form of Hebbian plasticity to serve as the neural mechanism for memory formation.

This exciting work opens several enticing new avenues of inquiry. One particularly interesting question will be to test whether different types of neurons play by different rules. In *Drosophila,* for example, different subpopulations of Kenyon cells are required at different times for memory acquisition and retrieval [16]. In addition, while the β-lobe neurons characterized by Cassenaer and Laurent [5] appear to be inhibitory, other neurons in the β-lobe may be excitatory; the properties and projection sites of all neurons of the

β-lobe remain to be revealed. The story of STDP within the mushroom body, already rich and complex, is just beginning.

It will be important to know how neuromodulators like octopamine affect other points within the olfactory system. Octopaminergic neurons are widely branching, extending into the antennal lobe and the mushroom body [17]. Although Cassenaer and Laurent [5] restricted application of octopamine to the β-lobe, it will be interesting to evaluate its other effects, which may be systemic, and may affect the ways output from the mushroom body is interpreted downstream. It will be interesting as well to explore mechanisms by which STDP tags synapses, particularly with respect to possibly analogous tagging mechanisms observed in mammals [18,19]. The recent development of a behavioral paradigm for assessing associative learning in locusts [20] will allow researchers to tackle these problems from physiology to behavior in a single animal. This will be an important step to understanding the formation of associative memories.

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Microtubule Organization: A Pericentriolar Material-Like Structure in Yeast Meiosis

During meiotic prophase in fission yeast, the nucleus undergoes dramatic oscillatory movements. A newly identified structure, the radial microtubule organizing center (rMTOC), mediates these movements and shares some of the features of the pericentriolar material in higher eukaryotes.

Alexander Dammermann[1], Lubos Cipak[1,2], and Juraj Gregan[1,*]

The microtubule cytoskeleton undergoes dramatic rearrangements during the cell cycle in order to create various specialized structures such as the mitotic spindle [1]. During prophase of fission yeast meiosis, microtubules are reorganized to form a single radial array associated with the spindle pole body (SPB), the yeast centrosome equivalent. This structure facilitates oscillatory movements of the nucleus, so-called horsetail movements, which have been shown to be important for meiotic recombination and proper segregation of chromosomes [2,3]. This process requires conversion of interphase microtubule bundles generated from multiple microtubule organizing centers (MTOCs) into a single radial microtubule (rMT) array. At the end of prophase, microtubules need to be reorganized again to allow formation of a bipolar spindle (Figure 1). How are these dramatic reorganizations of microtubule cytoskeleton accomplished? A new study published in this issue of *Current Biology* [4] shows that the transient generation of a novel microtubule organizing center called the radial microtubule organizing center (rMTOC) underlies formation of the radial microtubule array during meiotic prophase.

Performing EM tomographic reconstructions of cells undergoing meiotic prophase, Funaya *et al.* [4] made the exciting observation that microtubules do not emanate directly from the spindle pole bodies as previously thought, but rather from an electron-dense area located a distance of 30–180 nm away from the spindle pole body. They call this area the radial microtubule organizing center (rMTOC). This observation was unexpected because previous studies showed interphase microtubules to be located in close proximity to the spindle pole body [5]. What do we know about this rMTOC and how is it